METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF ISONIAZID AND PYRIDOXINE IN BULK FORM AND MARKETED TABLET DOSAGE FORMS BY RP-HPLC

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ABSTRACT: ***A simple, Accurate, precise method was developed for the simultaneous estimation of the Isoniazid and Pyridoxine in API form and Marketed pharmaceutical dosage form by RP-HPLC. Chromatogram was run through Phenomenex Luna C18 (4.6mm×150mm, 5µm) Particle size Column and Mobile phase containing Methanol and Tri Ethyl Amine Buffer taken in the ratio of 35: 65% v/v was pumped through column at a flow rate of 1.0 ml/min. Temperature was maintained at 38ºC. Optimized wavelength selected was 261 nm. Retention times of Isoniazid and Pyridoxine were found to be 2.256min and 5.427minutes respectively. The %RSD for the Repeatability and Intermediate Precision of the Isoniazid and Pyridoxine were found to be within limits. %Recovery was obtained was found to be within the limits for Isoniazid and Pyridoxine respectively. The LOD, LOQ values obtained from regression equations of Isoniazid and Pyridoxine were 2.63µg/ml and 3.84µg/ml & 7.92µg/ml and 11.54µg/ml respectively. Regression equation of Isoniazid and Pyridoxine was found to be y = 10511x + 9597.2 & y = 6120.9x + 29119 respectively. The Retention times was decreased and run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.***

Key Words:Isoniazid and Pyridoxine, RP-HPLC, Method Development, Validation, Accuracy.

I. INTRODUCTION

Isoniazide is a carbohydrazide obtained by formal condensation between pyridine-4-carboxylic acid and hydrazine. It has a role as an antitubercular agent and a drug allergen. It is functionally related to an isonicotinic acid. Isoniazid[1] is an antibacterial prescription medicine approved by the U.S. Food and Drug Administration (FDA) for the prevention and treatment of tuberculosis (TB). TB can be an opportunistic infection (OI) of HIV.Isoniazid is a bactericidal agent active against organisms of the genus Mycobacterium, specifically M. tuberculosis, M. bovis and M. kansasii. It is a highly specific agent, ineffective against other microorganisms[2]. Isoniazid is bactericidal when mycobacteria grow rapidly and bacteriostatic when they grow slowly.Isoniazid is a prodrug and must be activated by bacterial catalase. Specifically, activation is associated with reduction of the mycobacterial ferric KatG catalase-peroxidase by hydrazine and reaction with oxygen to form an oxyferrous enzyme complex. Once activated, isoniazid inhibits the synthesis of mycoloic acids, an essential component of the bacterial cell wall. At therapeutic levels isoniazid is bacteriocidal against actively growing intracellular and extracellular Mycobacterium tuberculosis organisms[3]. Specifically isoniazid inhibits InhA, the enoyl reductase from Mycobacterium tuberculosis, by forming a covalent adduct with the NAD cofactor. It is the INH-NAD adducts that acts as a slow, tight-binding competitive inhibitor of InhA. The IUPAC name of pyridine-4-carbohydrazide. The Chemical Structure of Isoniazid is shown in fig-1.

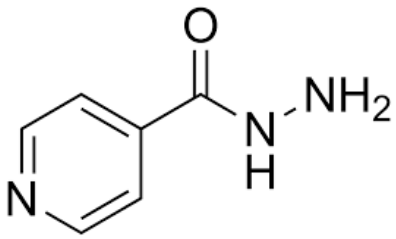

Fig.1. Chemical Structure of Isoniazid

Pyridoxine is a hydroxy methyl pyridine with hydroxymethyl groups at positions 4 and 5, a hydroxy group at position 3 and a methyl group at position 2. The 4-methanol form of vitamin B6, it is converted into to pyridoxal phosphate which is a coenzyme for synthesis of amino acids, neurotransmitters, sphingolipids and amino levulinic acid[4]. It has a role as a cofactor, a human metabolite, a Saccharomyces cerevisiae metabolite, an Escherichia coli metabolite and a mouse metabolite. It is a mono hydroxy pyridine, a vitamin B6, a member of methyl pyridines and a hydroxy methyl pyridine.Pyridoxine is indicated for the treatment of vitamin B6 deficiency and for the prophylaxis of Isoniazid-induced peripheral neuropathy[5]. It is also approved by Health Canada for the treatment of nausea and vomiting in pregnancy in a combination product with Doxylamine (as the commercially available product Diclectin).Vitamin B6 is the collective term for a group of three related compounds, pyridoxine (PN), pyridoxal (PL) and pyridoxamine (PM), and their phosphorylated derivatives, pyridoxine 5'-phosphate (PNP), pyridoxal 5'-phosphate (PLP) and pyridoxamine 5'-phosphate (PMP). Although all six of these compounds should technically be referred to as vitamin B6, the term vitamin B6 is commonly used interchangeably with just one of them, pyridoxine. Vitamin B6, principally in its biologically active coenzyme form pyridoxal 5'-phosphate, is involved in a wide range of biochemical reactions, including the metabolism of amino acids and glycogen, the synthesis of nucleic acids, hemoglobin, sphingomyelin and other sphingolipids, and the synthesis of the neurotransmitters serotonin, dopamine, norepinephrine and gamma-aminobutyric acid (GABA)[6]. The IUPAC name of Pyridoxine is 4,5-bis(hydroxymethyl)-2-methylpyridin-3-ol. The Chemical Structure of Pyridoxine is shown in follows

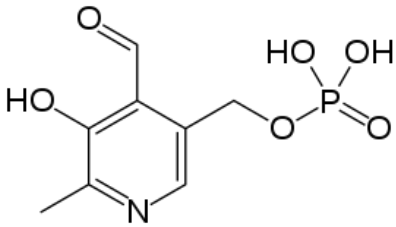

Fig.2. Chemical Structure of Pyridoxine

Literature review[36-38]reveals that the different HPLC and RP-HPLC methods havebeen developed individually or in combination withother drugs for both Isoniazid and Pyridoxine respectively. The aim ofthe current project was to develop a suitable methodfor the determination of simultaneous determinationof Isoniazid and Pyridoxine respectively.

II. MATERIALS AND METHODS

Table-1: Instruments used

S.No.	Instruments and Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module. 996 PDA detector, software: Empower 2
2	pH meter	LabIndia
3	Weighing machine	Sartorius
4	Digital ultra sonicator	Labman

Table-2: Chemicals used

S.No.	Chemical	Brand Names
1	Isoniazid	Local Market
2	Pyridoxine	Local Market
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck

HPLC Method Development:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Isoniazid and Pyridoxine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 1ml of the above Isoniazid and 3ml of Pyridoxine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Preparation of Sample Solution:

Take average weight of the Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight ofIsoniazid and Pyridoxine sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent[7].

Further pipette 1ml of Isoniazid and 3ml Pyridoxine above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure: Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Preparation of Mobile Phase:

Accurately measured 350ml (35%) of Methanol, 650ml of Tri Ethyl Amine Buffer (65%) were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration[8].

Diluent Preparation:

The Mobile phase was used as the diluent.

Method Validation Parameters

System Suitability

Accurately weigh and transfer 10 mg of Isoniazidand 10mg of Pyridoxine working sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1ml of Isoniazid and 3ml of Pyridoxine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

The sample solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Specificity:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Isoniazidand 10mg of Pyridoxine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1ml of Isoniazid and 3ml of Pyridoxine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution:

Take average weight of the Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight ofIsoniazid and Pyridoxine sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 1ml of Isoniazid and 3ml Pyridoxine above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Inject the three replicate injections of standard and sample solutions and calculate the assay[9] by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Linearity:

Accurately weigh and transfer 10 mg of Isoniazidand 10mg of Pyridoxine working sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Preparation of Level – I (60ppm of Isoniazid&100ppm of Pyridoxine):

Pipette out 0.6ml of Isoniazid and 1ml of Pyridoxine stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (80ppm of Isoniazid & 200ppm of Pyridoxine):

Pipette out 0.8ml of Isoniazid and 2ml of Pyridoxine stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (100ppm of Isoniazid & 300ppm of Pyridoxine):

Pipette out 1ml of Isoniazid and 3ml of Pyridoxine stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (120ppm of Isoniazid & 400ppm of Pyridoxine):

Pipette out 1.2ml of Isoniazid and 4ml of Pyridoxine stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (140ppm of Isoniazid & 500ppm of Pyridoxine):

Pipette out 1.4ml of Isoniazid and 5ml of Pyridoxine stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure:

Inject each level into the chromatographic system[10] and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Precision

Repeatability

Preparation of Isoniazid and PyridoxineSolution for Precision:

Accurately weigh and transfer 10 mg of Isoniazidand 10mg of Pyridoxine working sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1ml of Isoniazid and 3ml of Pyridoxine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

The sample solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate Precision:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:

Day 1:

The sample solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Day 2:

The sample solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits[11].

Accuracy:

For Preparation of 50% SampleStock Solution:

Accurately weigh and transfer 10 mg of Isoniazidand 10mg of Pyridoxine working sample into a 10ml of clean

dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).
Further pipette 0.5ml of Isoniazid and 1.5ml of Pyridoxine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

For Preparation of 100% SampleStock Solution:

Accurately weigh and transfer 10 mg of Isoniazidand 10mg of Pyridoxine working sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).
Further pipette 1ml of Isoniazid and 3ml of Pyridoxine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

For Preparation of 150% SampleStock Solution:

Accurately weigh and transfer 10 mg of Isoniazidand 10mg of Pyridoxine working Sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).
Further pipette 1.5ml of Isoniazid and 4.5ml of Pyridoxine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Isoniazidand Pyridoxine and calculate the individual recovery and mean recovery values[12].

Robustness:

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

For Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Isoniazidand 10mg of Pyridoxine working sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).
Further pipette 1ml of Isoniazid and 3ml of Pyridoxine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Effect of Variation of Flow Conditions:

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded

Effect of Variation of Mobile Phase Organic Composition:

The sample was analyzed by variation of mobile phase i.e. Methanol: Tri Ethyl Amine (35:65% v/v)was taken in the ratio and 40:60, 30:70 instead (35:65% v/v) remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

III. RESULTS AND DISCUSSION

Optimization of Method:

Optimized Chromatogram (Standard)

Mobile phase : Methanol: Tri Ethyl Amine Buffer (35:65% v/v)
Column : Phenomenex Luna C18 (4.6mm×150mm, 5µm) Particle size
Flow rate : 1 ml/min
Wavelength : 261 nm
Column temp : 38ºC
Injection Volume : 10 µl
Run time : 10 minutes

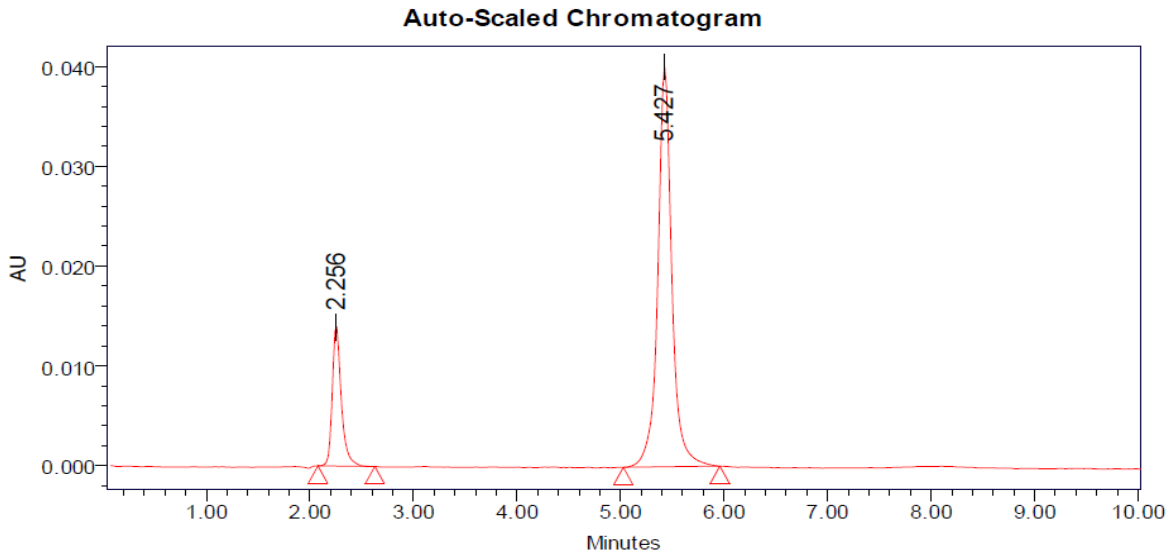

Fig.3. Optimized Chromatographic Condition

Validation of Analytical Method:

System Suitability:

Table-3: Results of System Suitability for Isoniazid

S.No.	Name	Rt	Peak Area	Height	USP Plate Count	USP Tailing
1	Isoniazid	2.247	105698	18652	7592	1.08
2	Isoniazid	2.246	105874	18754	7584	1.09
3	Isoniazid	2.248	105698	18698	7562	1.08
4	Isoniazid	2.252	105465	18689	7549	1.08
5	Isoniazid	2.248	105236	18695	7591	1.09
Mean			**105594.2**			
Std. Dev			**247.4049**			
% RSD			**0.234298**			

Table-4: Results of System Suitability for Pyridoxine

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Pyridoxine	5.452	1856985	63659	6359	1.05	5.86
2	Pyridoxine	5.484	1856754	63598	6384	1.04	5.85
3	Pyridoxine	5.491	1856985	63845	6395	1.05	5.86
4	Pyridoxine	5.482	1856574	63989	6345	1.04	5.86
5	Pyridoxine	5.491	1854735	63895	6395	1.05	5.85
Mean			**1856407**				
Std. Dev			**950.2696**				
% RSD			**0.051189**				

Specificity

The ICH documents[30,35]define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantitates Isoniazid and Pyridoxine in marketed formulation.

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Isoniazid and Pyridoxine in pharmaceutical dosage form (marketed formulation) was found to be 99.72%.

Linearity

Chromatographic Data for Linearity Study:

Table-5: Chromatographic Data for Linearity Study of Isoniazid:

Concentration µg/ml	Average Peak Area
60	648743
80	856982
100	1068542
120	1268984
140	1469853

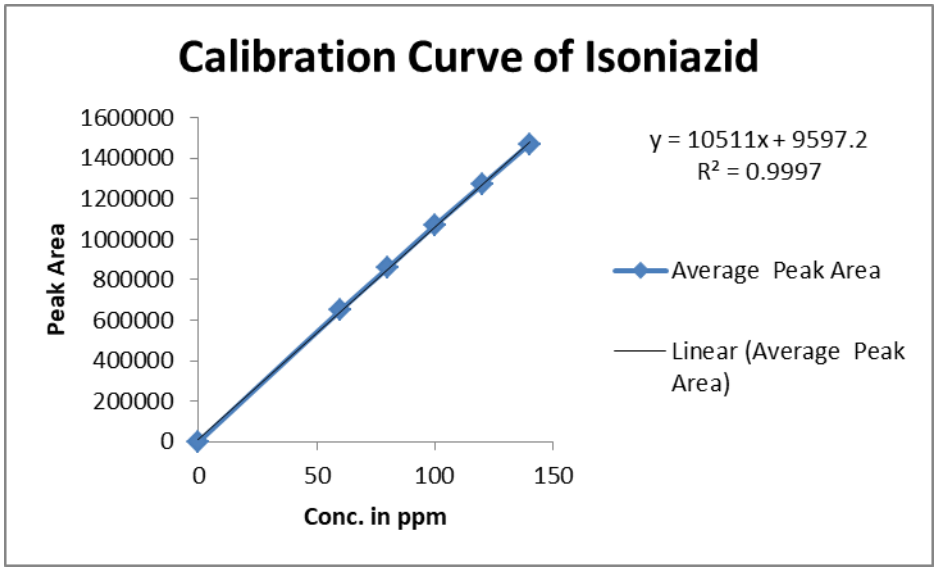

Fig.4. Calibration Graph for Isoniazid

Linearity Plot: The plot of Concentration (x) versus the Average Peak Area (y) data of Isoniazidis a straight line.

Y = mx + c

Slope (m) = 10511

Intercept (c) = 9597

Correlation Coefficient (r) = 0.999

Validation Criteria: The response linearity is verified if the Correlation Coefficient[13] is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 9597. These values meet the validation criteria.

Table-6: Chromatographic Data for Linearity Study of Pyridoxine

Concentration µg/ml	Average Peak Area
100	667564
200	1268547
300	1868598
400	2465487
500	3085864

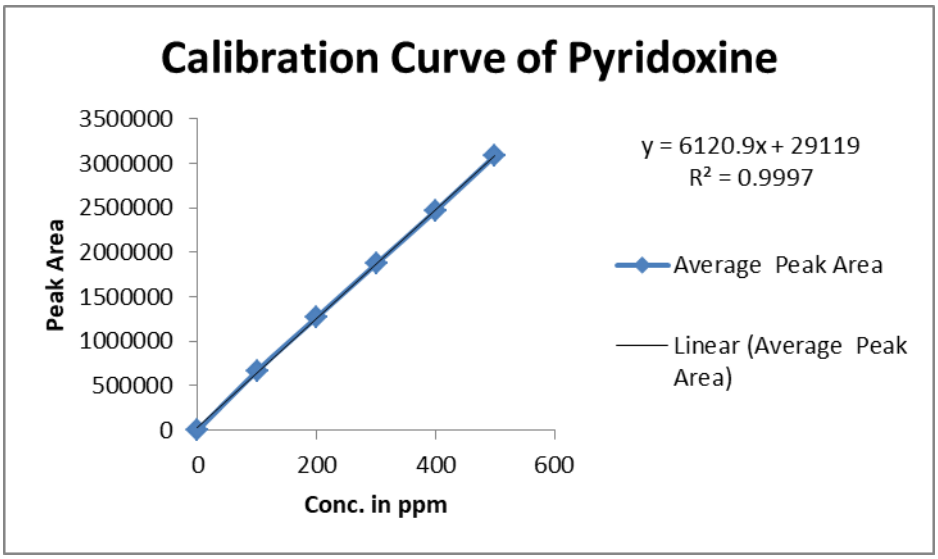

Fig.5. Calibration Graph for Pyridoxine

Linearity Plot: The plot of Concentration (x) versus the Average Peak Area (y) data of Pyridoxineis a straight line.

Y = mx + c
Slope (m) = 6120
Intercept (c) = 29119
Correlation Coefficient (r) = 0.999

Validation Criteria: The response linearity[14]is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 29119. These values meet the validation criteria.

Precision:

The precision[15] of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Repeatability

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions[16]. Recorded the peak areas and calculated % RSD.

Table-7: Results of Repeatability for Isoniazid:

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Isoniazid	2.269	105698	18569	7598	1.08
2	Isoniazid	2.255	105684	18547	7546	1.09
3	Isoniazid	2.252	105421	18594	7549	1.09
4	Isoniazid	2.267	105879	18574	7538	1.08
5	Isoniazid	2.260	105326	18563	7582	1.08
Mean			**105601.6**			
Std. Dev			**224.5023**			
% RSD			**0.212594**			

Table-8: Results of Method Precision for Pyridoxine

S. No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Pyridoxine	5.274	1856985	63598	6359	1.05	5.86
2	Pyridoxine	5.266	1857458	63579	6357	1.04	5.85
3	Pyridoxine	5.265	1854795	63547	6358	1.04	5.86
4	Pyridoxine	5.278	1857469	63592	6357	1.05	5.86
5	Pyridoxine	5.305	1857685	63569	6345	1.04	5.85

Avg			**1856878**				
Std. Dev			**1192.4**				
% RSD			**0.064215**				

Intermediate Precision:

Day 1:

Table-9: Results of Intermediate Precision for Isoniazid

S. No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Isoniazid	2.248	115246	19685	7698	1.09
2	Isoniazid	2.245	116985	19654	7685	1.09
3	Isoniazid	2.242	115847	19675	7645	1.09
4	Isoniazid	2.239	116985	19682	7682	1.09
5	Isoniazid	2.243	115848	19654	7691	1.09
6	Isoniazid	2.246	116582	19647	7642	1.10
Mean			**116248.8**			
Std. Dev			**710.3091**			
% RSD			**0.611025**			

Table-10: Results of Intermediate Precision for Pyridoxine

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Pyridoxine	5.284	1948592	64582	6459	1.05	5.96
2	Pyridoxine	5.293	1958245	64256	6475	1.06	5.95
3	Pyridoxine	5.306	1947584	64598	6498	1.05	5.96
4	Pyridoxine	5.319	1948675	64785	6472	1.06	5.95
5	Pyridoxine	5.346	1959854	64585	6493	1.05	5.96
6	Pyridoxine	5.352	1958246	64924	6438	1.06	5.96
Mean			**1953533**				
Std. Dev			**5792.661**				
% RSD			**0.296522**				

Day 2:

Table-11: Results of Intermediate Precision Day 2 for Isoniazid

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Isoniazid	2.255	102658	62584	6259	1.03
2	Isoniazid	2.260	102856	62359	6276	1.02
3	Isoniazid	2.242	102658	62451	6215	1.03
4	Isoniazid	2.245	102698	62584	6285	1.02
5	Isoniazid	2.260	102451	62758	6235	1.03
6	Isoniazid	2.255	102368	62154	6298	1.02
Mean			**102614.8**			
Std. Dev			**176.9592**			
% RSD			**0.17245**			

Table-12: Results of Intermediate Precision for Pyridoxine

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing	USP Resolution

1	Pyridoxine	5.266	1798952	62859	6265	1.03	5.42
2	Pyridoxine	5.265	1789854	62985	6289	1.02	5.43
3	Pyridoxine	5.306	1798659	62895	6279	1.03	5.42
4	Pyridoxine	5.293	1789898	62785	6285	1.02	5.43
5	Pyridoxine	5.265	1796856	62354	6249	1.03	5.42
6	Pyridoxine	5.266	1798568	62589	6245	1.02	5.43
Mean			**1795465**				
Std. Dev			**4390.879**				
% RSD			**0.244554**				

ACCURACY: Accuracy[17-20]at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

Table-13: The Accuracy Results for Isoniazid

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	539070	50	50.373	100.746%	100.36%
100%	1063578	100	100.274	100.274%	
150%	1587149	150	150.085	100.056%	

Table-14: The Accuracy Results for Pyridoxine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	949127	150	150.328	100.218%	100.15%
100%	1867824	300	300.441	100.147%	
150%	2785321	450	450.359	100.079%	

LIMIT OF DETECTION

The detection limit[21]of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

$$\mathbf{LOD = 3.3 \times \sigma / s}$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve[22]

Result:
Isoniazid:
=2.63µg/ml
Pyridoxine:
=3.84µg/ml

LIMIT OF QUANTITATION

The quantitation limit[23] of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

$$LOQ=10\times\sigma/S$$

Where
σ = Standard deviation of the response
S = Slope of the calibration curve

Result:
Isoniazid:
= 7.92µg/ml
Pyridoxine:
= 11.54µg/ml

ROBUSTNESS

The robustness[24] was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Isoniazid and Pyridoxine.

The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The samples (marketed formulation) of Isoniazid and Pyridoxine were injected by changing the conditions of chromatography[25-29]. There was no significant change in the parameters [31-34]like resolution, tailing factor, asymmetric factor, and plate count.

Table-15: Robustness for Isoniazid:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	105265	2.256	7589	1.08
Less Flow rate of 0.9 mL/min	109898	2.505	7256	1.05
More Flow rate of 1.1 mL/min	102365	2.046	7469	1.07
Less organic phase	101548	2.505	7358	1.06
More organic phase	104645	2.046	7659	1.02

Table-16: Robustness for Pyridoxine:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1858475	5.427	6354	1.04
Less Flow rate of 0.9 mL/min	1925684	5.599	6253	1.05
More Flow rate of 1.1 mL/min	1863525	4.576	6248	1.03
Less organic phase	1825471	5.599	6415	1.02
More organic phase	1836594	4.576	6529	1.06

IV. SUMMARY AND CONCLUSION

SUMMARY

In the present study the analytical method is developed for analysis of Isoniazid and Pyridoxine in bulk form and marketed pharmaceutical dosage form by using RP-HPLC. Analytical method was developed for analysis of

Isoniazid and Pyridoxinein bulk form and marketed pharmaceutical dosage form by using Phenomenex Luna C18 (4.6mm×150mm, 5µm) Particle size and of Methanol: Tri Ethyl Amine Buffer in the ratio of 35:65% v/vused as mobile phase at 1.0 mL/min. The UV detector wave length is 261 nm. The developed method is economically feasible than RP-HPLC method, reproducible, selective, precise, specific and accurate than existing methods. This method can be used as alternative for HPLC methods.

V. CONCLUSION

The advantages of the proposed method involve a simple procedure for samplepreparation and relatively short time of analysis. Apart from this, it can be used for assays of Isoniazid and Pyridoxine in bulk forms or in pharmaceutical formulations. The proposed method was validated by testing its linearity, accuracy, precision, limits of detection, and limit of quantitation. Robustness and stability of solutions. The results of the analysis of pharmaceutical dosage forms by the proposed methods are highly reproducible, reliable, and are in good agreement with the label claims of the drug. The additives usually present in the pharmaceutical formulations of the assayed samples did not interfere with Isoniazid and Pyridoxine. It may be said that the proposed methods are precise, sensitive, and accurate, so that these can be used as standard Pharmacopoeial methods for the determination of Isoniazid and Pyridoxine using the RP-HPLC systems with PDA detector.

REFERENCES

1. https://go.drugbank.com/drugs/DB00951
2. https://pubchem.ncbi.nlm.nih.gov/compound/Isoniazid
3. https://en.wikipedia.org/wiki/Isoniazid
4. https://go.drugbank.com/drugs/DB00165
5. https://pubchem.ncbi.nlm.nih.gov/compound/1054
6. https://en.wikipedia.org/wiki/Pyridoxine
7. Ahuja S. In: High Pressure Liquid Chromatography of Comprehensive Analytical Chemistry. Elsevier Publications. 2006.
8. Synder LR, Kirkland JJ and Glajch JL. In: Practical HPLC Method Development, 2nd Ed, John Wiley and Sons Inc. Canada. 1997.
9. Settle FA, In: Handbook of Instrumental Techniques for Analytical Chemistry. 1st Ed, Singapore, Pearson Education Inc.2004.
10. Mohammad T et al., HPLC Method Development and Validation for Pharmaceutical Analysis- A Review. International Pharmaceutica Sciencia. 2012, 2(3), 14.
11. British Pharmacopoeia.(International Ed.) 1993; Vol. 1:429, 483. Published on the Recommendation of the Medicines Commissions Pursuant to Medicines Act 1968, 1993.
12. United States Pharmacopoeia 29 NF 24, Published on the Recommendation of the Medicines Commissions Pursuant to Medicines, page no. 587.
13. Dr. Kealey and P.J Haines, Analytical Chemistry, 1st edition, Bios Publisher, (2002), P1-7.
14. "HPLC in Pharmaceutical Industry", Fong and Long, Marcel Dekker Series
15. "Instrumental Method of Chemical Analysis" by Chatwal Anand, Himalaya Publishing House, p.no.615-623.
16. "Practical Pharmaceutical Chemistry", 4th edition, Part 2, by Beckett and Stenlake, CBS Publishers and Distributors, P.No.157-174.
17. Vibha G et al., Development and validation of HPLC method - a review. International Research Journal of Pharmaceutical and Applied Sciences. 2012, 2(4), 22-23.
18. Validation of Analytical Procedures: Methodology. ICH-Guidelines Q2B, Geneva. 1996, 11. (CPMP/ICH/281/95).
19. Development and validation of HPLC method - A Review, Vibha Gupta et al, International Research Journal of Pharmaceutical and Applied Sciences, 2012; 2(4):17-25.
20. A Review: HPLC Method Development and Validation, Santosh Kumar Bhardwaj *et al. International Journal of Analytical and Bioanalytical Chemistry, accepted 20 November 2015.
21. Method Development: A Guide to Basics Quantitative & Qualitative HPLC, LC, GC chromacademy.
22. Santhosh Illendula, G. Laxmi prasanna ; Simultaneous estimation of new analytical method development & validation of Doxylamine & Pyridoxine by HPLC in bulk & marketed formulation, WJPPS 08(10) 2019, 973-1004.
23. Santhosh Illendula, G. MariyaTherisa ;Simultanious estimation of latest analytical method improvement and validation of Dabrafenib & Trametinib by HPLC , WJPPS 10(3) 2021, 1978-2004.
24. Santhosh Illendula, S Joythi& K rajeswarDutt ; Development and validation of an RP HPLC method for the determination of riociguat in pharmaceutical dosage form, Int. J of research 10(2) Feb 2021 ,344-357.
25. Lalit V Sonawane* Bioanalytical Method Validation and Its Pharmaceutical Application- A Review Pharmaceutica Analytical Acta 2014, 5:3Center for Drug Evaluation and Research (CDER) Reviewer Guidance.
26. ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology.
27. T.Benjamin1*, D. Ramachandran2, Development and Validation of RP-HPLC Method for Simultaneous Estimation of Isoniazid & Pyridoxine in Bulk and It Pharmaceutical Formulations, American Journal of PharmTech Research, 2019; 9(01), Pages: 215-225.
28. S. Khatak, Mamta Khatak, and F. Ali1, Ashu Rathi1, R. Singh1, G. N. Singh1 and H. Dureja*, Development and Validation of a RP-HPLC Method for Simultaneous Estimation of Antitubercular Drugs in Solid Lipid Nanoparticles, Indian J Pharm Sci 2018; 80(6): 996-1002.
29. Santhosh Illendula, K. Sai Sneha & Rajeswar Dutt ; A new RP HPLC method for the simultaneous estimation of Atazanavir and Ritonavir in its pure and pharmaceutical dosage form as per ICH guidelines , WJPPS 08(09) 2019, 1018-1033.
30. S.K. Dhal and R. Sharma*, Development and Validation of RPHPLC Method for Simultaneous Determination of Pyridoxine Hydrochloride, Isoniazid, Pyrazinamide and Rifampicin in pharmaceutical Formulation, Chemical Analysis, (Warsaw), 54, 1487 (2009).